HIV-1: Packaging a Shifty Genome?

Michael A. Durney[1] and Victoria M. D'Souza[1,*]
[1]Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138, USA
*Correspondence: dsouza@mcb.harvard.edu
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In this issue of *Cell Host & Microbe*, Chamanian et al. (2013) show that the frameshifting region in the HIV-1 genome influences the efficiency of genome packaging. This study may provide insights into mechanisms that constrain retroviruses into packaging only two copies of the genome during retroviral assembly.

The mechanism by which retroviruses selectively package their RNA genomes has been a topic of intensive research for more than three decades. Retroviruses are unique in that their genomes are made up of two copies of the full-length, unspliced mRNA. This genomic mRNA—transcribed by the host cellular machinery with reverse-transcribed viral DNA as a template—is also used for translating the essential Gag and Gag-Pol viral proteins (D'Souza and Summers, 2005). The virion is thus required to efficiently discriminate its genome from a pool of excess cellular mRNA prior to budding.

A region called the packaging signal (Ψ site) present in the 5′ untranslated region (5′UTR) of the mRNA is known to dictate this specificity (Figure 1). Specific sequences in the Ψ site are capable of binding to the nucleocapsid (NC) domain of the viral Gag protein, thus allowing the genome to be integrated into assembling virions. Furthermore, the Ψ site also typically contains dimerization determinants, enabling the virion to encapsidate a diploid genome (D'Souza and Summers, 2005).

Recent work in HIV-1 suggests that the dual function (translational versus genomic) and the dual state (monomeric versus dimeric) of the viral RNA may be correlated. Briefly, the Ψ site of the mRNA can be maintained in two conformations: the monomeric form has the NC-binding sequences and dimerization initiation site (DIS) sequestered and the Gag start codon exposed, which makes the mRNA translation competent, while a switch to the dimeric conformation sequesters the stop codon and exposes the NC-binding site and makes the mRNA competent for packaging (Lu et al., 2011). In HIV-1 it is thus currently believed that there exists a single pool of mRNA that can be driven by the Ψ site to switch between equilibrating conformations, which in turn determines the immediate fate of the mRNA (Lu et al., 2011; Butsch and Boris-Lawrie, 2002).

While the critical role of the Ψ site as the major determinant for genome dimerization and encapsidation has been conclusively established, additional contributions from sequences in the 5′UTR near the Ψ site, including the TAR, Poly(A), and U5-PBS domains, as well as sequences further downstream into the Gag coding region, have been reported (Heng et al., 2012). All of these contributions, however, have been questioned by additional research. One of the reasons for inconsistencies arises from the fact that these domains play an important role in other functions that are critical for retroviral replication. In addition, mutations of these distinct domains are capable of perturbing the Ψ site structure. Thus, in these cases, the observed defects in genome packaging could be an indirect or secondary effect. In fact, recent studies that ensure maintenance of the correct three-dimensional structure of the Ψ site have concluded that these additional domains do not significantly contribute to packaging specificity and levels (Heng et al., 2012).

There are, however, key questions that remain unanswered in the field. For example, why are only two genomes packaged per virion? Are there other determinants present in the viral mRNA itself, or viral proteins and/or host factors that allow for only a fraction of the mRNA to become competent for packaging and thus limit the number the genomes packaged per virion?

A new study by Chamanian and colleagues in this issue of *Cell Host & Microbe* (Chamanian et al., 2013) suggests that a sequence even further downstream into the HIV-1 mRNA-coding region, specifically at the Gag-Pol junction, contributes to packaging (Figure 1). But unlike the other domains that were speculated to only contribute to the packaging efficiency, this study suggests that the region may hold clues to the above open questions. The authors start by defining the domain as a "genomic RNA packaging enhancer" (GRPE) after finding that deletion of this sequence from a wild-type construct, containing an intact 5′UTR, causes a 7-fold decrease in packaging efficiency. The authors then perform in vitro SHAPE analysis (a chemical probing method that maps secondary structure) of the GRPE in RNA constructs designed to change the spacing between 5′UTR and the GRPE. They find that constructs that cause perturbation of the GRPE structure also cause a decrease in genome packaging.

Interestingly, the GRPE contains a frameshifting signal that dictates the ~5% ribosomal recoding frequency in HIV-1. Recoding, either by frameshifting (in HIV-1) or stop codon readthrough (as in murine leukemia virus, MLV) is required for the production of the critical 20:1 Gag to Gag-Pol protein ratio in retroviruses. To probe for a possible connection between frameshifting frequencies and genome packaging, the authors sought to manipulate frameshifting levels by knocking down eRF1, a host factor that has been reported to modulate frameshifting (Kobayashi et al., 2010). In this scenario, the authors observe a 10-fold increase in genomes being packaged per Gag molecule, i.e., ~20 genomes are packaged per virion. The authors then use previously observed correlations to the nonsense-mediated mRNA decay (NMD) pathway to describe a model for their findings. First, eRF1 is directly implicated in NMD, and hence bypassing the stop codon during

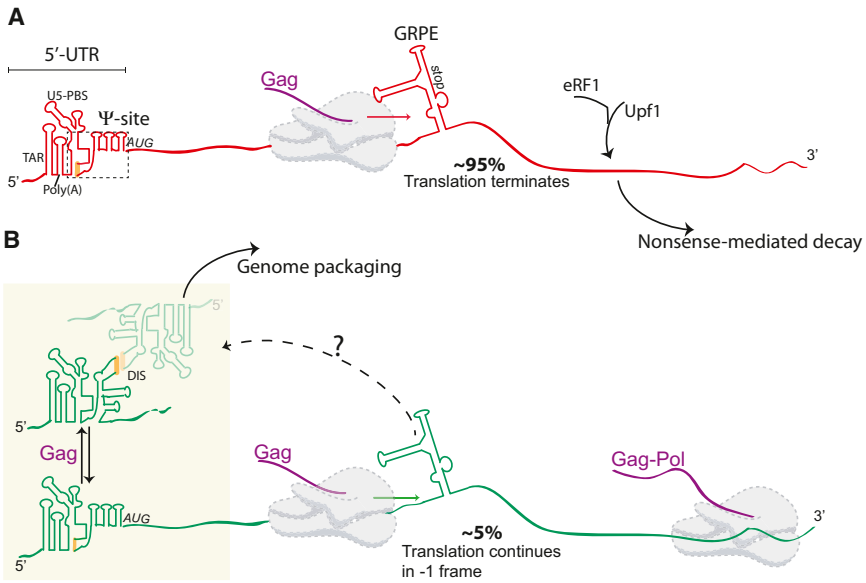

Figure 1. Potential Relationship between Ribosomal Frameshifting and Genome Packaging in HIV-1

(A) Approximately ninety-five percent of the viral mRNA terminates at the stop codon after translating the Gag protein. The 5′UTR consisting of the Ψ site, TAR, Poly(A), and U5-PBS is in the translation-competent conformation with the DIS (orange) sequestered. Recruitment of eRF1 and Upf1 designates this fraction of the mRNA for NMD.

(B) Only 5% of the viral mRNA undergoes frameshifting and continues translation in the −1 frame, thus bypassing the stop codon and translating the Gag-Pol fusion protein. This fraction of the mRNA may be protected from NMD, allowing for a potential long-range interaction between the GRPE and the dimerized Ψ site, which in turn facilitates dimeric genome packaging.

frameshifting may indeed protect this fraction of mRNA from NMD. Second, the recoding signal itself (at least in MLV) has been shown to inhibit decay by blocking RNA binding of Upf1, which is a nonspecific RNA helicase component of the NMD surveillance complex (Hogg and Goff, 2010). The authors suggest that after Gag translation the interactions of eRF1, Upf1, and associated NMD factors may preclude formation of long-range interactions between the GRPE and the Ψ site. When frameshifting does occur, eRF1 and the NMD factors are dissociated from the RNA by translating ribosomes potentially allowing the formation of long-range interactions necessary for packaging. Thus, the authors speculate that only 5% of mRNA that allow for frameshifting may be resistant to NMD and therefore destined for packaging (Figure 1).

From the current study it is not clear if the regulatory effect of the GRPE on packaging arises due to direct contacts between the frameshifting signal and the 5′UTR. It has recently been shown that the frequency of recoding in MLV is regulated by a dynamic, preexisting, structural equilibrium between two conformations of the recoding signal: an active conformer that is permissive for recoding, and an alternate, inactive conformer that terminates translation (Houck-Loomis et al., 2011). It is possible that HIV-1 frameshifting frequency is also maintained by multiple conformations, each having a distinct fate, but this possibility has yet to be investigated. Since change in local structure can have long-range effects, it is possible that the same riboswitch that induces a frameshifting permissible conformation also leads to the packaging competent structure in the 5′UTR, thus linking the two processes without necessitating a direct interaction between the frameshifting signal and the 5′UTR. In fact, long-range influences from TAR-Poly(A) in the 5′UTR have been shown to influence the efficiency of the distally located frameshifting signal by interfering with translation initiation (Charbonneau et al., 2012).

Linking eRF1 levels to packaging is intriguing because this observation potentially links regulation of ribosomal translation to genome packaging. However, pulse-chase experiments have shown that inhibition of translation in HIV-1-infected T cells does not affect RNA packaging, and hence de novo translation is not thought to be a requirement for packaging in HIV-1 (Butsch and Boris-Lawrie, 2002). The new model is thus a bold departure and, as the authors note, warrants further investigation to determine the exact cause for deregulation of diploid genome packaging upon eRF1 downregulation. An important experiment will be to correlate packaging levels directly with frameshifting levels. The sequence requirements for the HIV-1 frameshifting signal have been extensively studied by mutagenesis (Mouzakis et al., 2012). It should, in theory, be possible to investigate whether modulation of frameshifting directly leads to the predicted effects on packaging. However, mutations known to abrogate recoding in MLV are still able to protect the mRNA from NMD (Hogg and Goff, 2010), suggesting that recoding signal does not need to have an active conformation to exert its effect. Thus, in order to directly correlate frameshifting levels with genome packaging, hyperactive frameshifting mutants may be required (Mouzakis et al., 2012). This should, in principle, lead to a greater fraction of mRNA that escapes NMD and hence allow for packaging of more genomes. Furthermore, advances in NMR spectroscopy using specific labeling techniques and emerging high-resolution structural data may now allow direct probing of putative interactions between the GPRE and the Ψ site (Lu et al., 2011).

The findings of this study are exciting and may finally set the stage for understanding critical regulatory events that lead to diploid retroviral genome packaging.

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Placental Malaria: From Infection to Malfunction

Luciana Vieira de Moraes[1] and Carlos Penha-Gonçalves[1,*]
[1]Instituto Gulbenkian de Ciência, Rua da Quinta Grande, 6, 2780-156 Oeiras, Portugal
*Correspondence: cpenha@igc.gulbenkian.pt
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Malaria during pregnancy is a major factor in infant morbidity and mortality. In this issue of *Cell Host and Microbe*, Conroy et al. (2013) propose that C5a, a product of complement cascade activation, counteracts the placental vascular remodeling response induced by *Plasmodium* infection and contributes to fetal growth restriction.

In Māori tradition, the placenta (*whenua* in the language of Māori) is buried upon birth to establish a link between the land (likewise called *whenua*) and the newborn. Both types of *whenua* nourish: "One allows the foetus to become a baby with all the potential to become a strong and healthy adult, and the other sees that person develop and grow, make their contribution to society and then be 'born' into the spirit of the world" (Head and Head, 2003). Although the placenta is credited with the developing fetus, surprisingly little is known about critical molecules and physiological parameters that control the functional efficacy of this fast-growing organ and determine pregnancy success.

Fetal growth restriction is a severe consequence of malarial disease during pregnancy and thought to result largely from placental insufficiency. Much research has been centered on the pathogenic role of ligand-receptor interactions between the *Plasmodium*-infected erythrocyte and placental tissues (reviewed by Duffy and Fried, 2005). Parasite antigens expressed on the surface of infected erythrocytes are thought to contribute to accumulation of parasitized cells in the placenta. The best example is var2csa, member of the *var* (variable surface antigen) gene family that is selectively expressed by infected erythrocytes that accumulate in the placenta (Salanti et al., 2003). VAR2CSA has strong affinity to the glycosaminoglycan chondrotin sulfate A (CSA or C4S) present in high amounts in the intervillous space and on the surface of syncytiotrophoblasts, which contact the maternal blood. The current pathogenesis model is focused on linking placental sequestration of infected erythrocytes to a series of inflammatory events that can reduce fetal growth. Cytoadhesion of infected erythrocytes can activate syncytiotrophoblast cells to secrete chemokines, which attract maternal inflammatory cells (Lucchi et al., 2008). But how does inflammation impair maternal-fetal exchange? Little attention has been paid to the impact of malaria on physiology of the fast-developing placenta that in the course of infection may contribute to placental insufficiency. Recently the pathogenesis paradigm was challenged by work that addresses the role of angiogenesis factors (reviewed by Conroy et al., 2011), the coagulation cascade (Avery et al., 2012), and placental microcirculation (Moraes et al., 2013).

In this issue, Conroy et al. (2013) investigate potential links between inflammation, angiogenesis alterations, and fetal growth restriction in pregnancy-associated malaria. They focus on activation of the complement cascade, previously shown to impair placental vascular remodeling in mouse models of miscarriage or fetal growth restriction (reviewed by Girardi, 2008). Complement cascade activation by immune complexes in the placenta may indirectly contribute to inflammation. C5a—a protein fragment released by the activation of C5 complement component—was shown to be implicated in tissue injury by inducing release of reactive oxygen species of C5a-activated neutrophils. Nevertheless, in antibody-independent models of unsuccessful pregnancy, inflammatory cells indirectly generate C5a fractions that activate monocytes to produce soluble vascular endothelial growth factor (sVEGF), an inhibitor of VEGF. This deregulation of angiogenic factors is hypothesized to disturb the developing placental vascularization and as a consequence impeding babies of getting appropriate nurturing.

The study by Conroy et al. (2013) tackles this issue and provides epidemiological data and compelling experimental